APPEARANCE OF MULTIRESISTANT *SALMONELLA* TYPHIMURIUM DT104 IN SWINE IN POLAND

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Summary: *Salmonella* (*S.*) Typhimurium phage type distribution remains unrecognised in Poland. We describe DT104 that appeared in swine no later than in 1998. The study revealed the presence of two integrons of 1.0 kb and 1.2 kb referring to, aminoglycoside and b-lactamase resistance genes cassette respectively in all DT104 and in one NT strain showing ACSSuT profile. These isolates tend to demonstrate additional resistance profiles. The findings illustrate the world-wide spread of multiresistant clone and point out the import of subclinically infected animals as a source of DT104 introduction into Polish swine herds.
Keywords: pigs, salmonellosis, antibiotic resistance, integrons

Materials and Methods: The study included *S.* Typhimurium strains isolated during 1994-2002 (n=20) and 1979-1988 (n=8) from clinical samples of diseased pigs. Four of the isolates originated from imported animals. MIC was determined with microbroth two-fold dilution method (Sensititre) and interpreted according NCCLS guidelines. The strains were phagetyped according to Callow's system (Anderson E.S. et al., 1977). Integron detection by PCR using the primers att1-F and 3_CS-B, were performed as described by Sandvang et al. (Sandvang D, Diggle M Platt DJ, 2002). Contingency test (Yates's correction) at the 0.05 level of significance was used for statistical purposes.

Results: *S.* Typhimurium characteristics were gathered in table 1. No apramycin, gentamicin, colistin, ceftiofur, amoxicillin/ clavulanic acid and ciprofloxacin resistance was found. Nine isolates were sensitive to all antimicrobials tested and belonged to 7 different phage types or showed unspecific reactions (RDNC). Resistance was more frequently found in isolates from 1994-2002 ($p \Theta 0.01$) and DT110 and DT104 predominated amongst five phage types detected. Tetracycline-resistance as a single resistance determinant was observed exclusively in DT110 and DT208 isolates ($p \Theta 0.01$). Two DT110 and 1 NT strain showed ASSuT profile. ACSSuT resistance was observed in 6 DT104 and 1 NT strain that revealed also the presence of PCR products corresponding to the resistance gene cassette.

Discussion: *S.* Typhimurium is either the prevalent or the second *Salmonella* serovar most frequently found in swine (Baggesen, D. L., Sandvang, D., and Aarestrup, F. M., 2000;Hoszowski A. and Wasyl D., 2002). Despite the importance of *S.* Typhimurium as a cause of swine infections, the phage type distribution in Poland remains unknown. Therefore the aim of the research was to confirm the appearance of *S.* Typhimurium DT104 in swine in Poland and determine the presence of class 1 integrons. DT104 has not yet been reported in Poland and this paper should be considered as the first report on this phage type with integron mediated multi-resistance in animals. The first strain was obtained in 1998 and since then DT104 has been isolated from swine salmonellosis. It was found in imported animals introduced into breeding herds. All ACSSuT strains revealed the presence of 1.0 kb and 1.2 kb PCR products corresponding to two common integrons of the resistance gene cassettes (Baggesen, D. L., Sandvang, D., and Aarestrup, F. M., 2000;Lawson, A. J. et al., 2002;Sandvang D, Diggle M Platt DJ, 2002). Integron positive nontypable strain was due to decreased phage sensitivity rather than appearance of multi-resistance genes in non-DT104 isolates (Lawson, A. J., Dassama, M. U., Ward, L. R., and Threlfall, E. J., 2002). Integron-positive *S.* Typhimurium strains had additional resistance determinants (Baggesen, D. L., Sandvang, D., and Aarestrup, F. M., 2000). Resistance profiles noted in present study

gathered up to 10 antimicrobials. DT104 strains were often nalidixic acid resistant (p Θ 0.001). Although florfenicol, neomycin and trimethoprim joined the basic penta-resistant profile, no significance was noted in respect of those antimicrobials. The findings illustrate the world-wide clonal spread of multi-resistant *S.* Typhimurium DT104.

Conclusions: Penta-resistant *S.* Typhimurium DT104 has been present in Polish swineherds since at least 1998. The possible source of introduction of the clone to Poland was imported symptomless, chronically infected animals.

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Table 1. *S. Typhimurium antibiotic susceptibility, R-types ([-] – susceptible strain), phage types and presence of integrons (n – not detected, p – detected)*

Strain No.	Year	MIC (μg/ml)																	R-types	Phage types	Integrons
		A/C	A	Apr	Cft	C	Cip	Col	Ff	Gen	Na	N	Sp	S	Su	T	Tm	T/S			
2416	1979	2	1	4	0,5	4	0,03	4	2	1	4	2	32	8	32	2	4	1	[-]	DT66	n
2466	1979	2	1	4	0,5	2	0,03	4	4	1	4	2	128	32	512	32	32	8	SSuTSpT/STm	DT135	n
2468	1979	2	1	4	0,5	8	0,03	4	4	1	4	2	32	8	32	32	4	1	T	DT110	n
15	1980	2	1	4	0,5	4	0,03	4	2	1	8	2	32	16	32	2	4	1	[-]	DT37	n
471	1985	2	1	4	0,5	4	0,03	4	2	1	4	2	32	8	64	2	4	1	[-]	RDNC	n
501	1985	2	1	4	0,5	4	0,03	4	4	1	8	2	32	8	32	2	4	1	[-]	U275	n
592	1985	2	1	4	0,5	2	0,03	4	2	1	4	2	16	8	32	2	4	1	[-]	DT9	n
92	1988	2	1	4	0,5	2	0,03	4	2	1	4	2	32	4	32	2	4	1	[-]	DT135	n
491	1994	2	2	4	0,5	4	0,03	4	2	1	4	2	32	8	32	2	4	1	[-]	RDNC	n
606	1996	2	2	4	0,5	4	0,03	4	4	1	4	2	32	8	32	32	4	1	T	DT208	n
607	1996	2	2	4	0,5	4	0,03	4	4	1	4	2	32	8	32	32	4	1	T	DT208	n
609	1996	2	2	4	1	4	0,03	4	4	1	4	2	32	8	32	32	4	1	T	DT208	n
118	1998	16	32	4	0,5	64	0,03	4	16	1	4	2	128	64	512	16	4	1	ACSSuTSp	DT104	p
1102	2000	2	1	4	0,5	4	0,03	4	4	1	4	2	32	8	32	2	4	1	[-]	DT41	n
1103	2000	8	32	4	0,5	8	0,03	4	4	1	4	2	32	64	512	32	4	1	ASSuT	DT110	n
216	2000	16	32	4	1	64	0,25	4	32	1	128	2	128	64	512	16	4	1	ACSSuTSpNaFf	DT104	p
425	2000	4	32	4	0,5	8	0,03	4	4	1	8	2	32	64	512	32	4	1	ASSuT	NT	n
816	2000	16	32	4	0,5	64	0,25	4	16	1	128	2	128	32	512	32	4	1	ACSSuTSpNa	DT104	p
190	2001	16	32	4	1	64	0,03	4	32	1	4	2	128	64	512	32	4	1	ACSSuTSpFf	NT	p
191	2001	2	1	4	0,5	4	0,03	4	4	1	4	2	32	8	32	32	4	1	T	DT110	n
192	2001	2	1	4	0,5	8	0,03	4	4	1	4	2	32	8	32	32	4	1	T	DT110	n
193	2001	2	1	4	1	8	0,03	4	4	1	4	2	32	8	32	32	4	1	T	DT110	n
194	2001	2	1	4	0,5	8	0,03	4	4	1	4	2	32	8	32	32	4	1	T	DT110	n
215	2001	8	32	4	0,5	8	0,03	4	4	1	4	2	32	64	512	32	4	1	ASSuT	DT110	n
298	2001	16	32	4	0,5	64	0,25	4	64	1	128	2	128	64	512	32	4	1	ACSSuTSpNaFf	DT104	p
323	2001	16	32	4	0,5	64	0,25	4	16	1	128	32	128	64	512	32	32	8	ACSSuTNSpNaT/STm	DT104	p
618	2001	2	1	4	0,5	4	0,03	4	2	1	8	2	32	16	32	2	4	1	[-]	DT8	n
7	2002	16	32	4	0,5	64	0,25	4	16	1	128	2	128	32	512	16	4	1	ACSSuTSpNa	DT104	p